Preventive Effects of 10-Day Supplementation With Saffron and Indomethacin on the Delayed-Onset Muscle Soreness

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**Objective:** Delayed-onset muscle soreness (DOMS) often occurs after unaccustomed eccentric exercise and reduces exercise performance. We aimed to study the preventive effects of saffron and indomethacin on the biochemical and functional indicators of DOMS after 1-session eccentric exercise.

**Design:** A 10-day, randomized, double-blind, placebo-controlled, pretest–posttest design.

**Setting:** Controlled research laboratory.

**Participants:** Thirty-nine nonactive male university students randomly divided into saffron (n = 12), indomethacin (n = 12), and control (n = 15) groups.

**Interventions:** Saffron group received 1 capsule containing dried saffron powder (n = 12, 300 mg/d), indomethacin group received 75 mg indomethacin (n = 12, 25 mg thrice a day), and control group (n = 15) received placebo capsules, 1 week before and 3 days after eccentric exercise. Ten days before and 24, 48, and 72 hours after muscle soreness protocol, the maximum isometric and isotonic forces, plasma creatine kinase (CK), plasma lactate dehydrogenase (LDH), perceived pain, knee range of movement, and thigh circumference were measured. Muscle soreness protocol was performed with a weight load equal to 80% of the maximum isotonic force in 4 sessions with 20 repetitions and 3-minute rest in between.

**Main Outcome Measures:** This study shows that 10-day supplementation with 300 mg saffron significantly decreased the CK and LDH concentrations (P < 0.0001). In the saffron group, there was no decline in maximum isometric and isotonic forces after eccentric exercise, but a significant decline in the isometric force was observed in the control group (P < 0.0001). No pain was reported in the saffron group, whereas the indomethacin group experienced pain before 72 hours (P < 0.001).

**Conclusions:** Results obtained from the current novel research indicate a strong preventive effect of 10-day supplementation with saffron on the DOMS.

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**Clinical Relevance:** The saffron can be used to prevent DOMS and alleviate the DOMS symptoms.

**Key Words:** saffron, indomethacin, delayed-onset muscle soreness, creatine kinase, lactate dehydrogenase, isometric and isotonic forces


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**INTRODUCTION**

Delayed-onset muscle soreness (DOMS) is associated with pain and discomfort in the first few days after a strenuous exercise session. Eccentric muscular contractions in downhill running, hopping, plyometric exercise, squatting, and during the lowering phase of lifting weights can produce DOMS. Athletes are concerned about muscular discomfort and pain phenomena because it can limit their exercise and training activity. The main symptoms in DOMS are muscular stiffness, tenderness, and pain during active movements. There are many symptoms related to the muscle inflammation and damage such as muscle fibre swelling, elevated serum activities of muscle specific enzymes such as the creatine kinase (CK) and lactate dehydrogenase (LDH), reduced muscle strength, and knee joint range of movement (ROM).

Eccentric exercise has series of effects in the cell membrane, causing an inflammatory response that leads to production of prostaglandin E2 and leukotrienes. The muscle microscopic injury is instigated by a mechanical disruption to sarcomeres, T-tubules, myofibrils, cytoskeletal protein, and the sarcoplasmic reticulum, which leads to an inflammatory response. After muscle injury, enzymatic reactions and inflammatory mediators such as thromboxanes, prostaglandins, and leukotrienes from the cyclooxygenase and lipoxygenase pathways correspond to increases in vascular permeability and pain perception by sensitizing the types III and IV afferent nerve fibres to both chemical and mechanical stimuli.

The magnitude of force loss after eccentric exercise has been claimed to be the best indirect marker of muscle soreness. Mechanism of maximal force reduction in DOMS is thought to be secondary to sarcomere “popping” and disorganization, as well as damage to components of the excitation–contraction coupling process. Prevention and treatment strategies to alleviate the symptoms and signs of DOMS are numerous and varied, including pharmacological (eg, nonsteroidal anti-inflammatory medications), exercise, stretching, whey protein, fish oil, isoflavones, caffeine, l-carnitine, herbs, antioxidant vitamins, cryotherapy, transcutaneous electrical nerve stimulation, and ultrasound. However, these...
methods had small or no effect on DOMS or were not consistent in attenuating DOMS pain and other symptoms.

Administration of aspirin and acetaminophen did not reduce the DOMS and the CK response to eccentric exercise. Preclinical studies found that ibuprofen has no effect on muscle soreness. In addition, several dietary supplements have been tested in the treatment of DOMS, including whey protein, vitamin C, protease enzymes, phosphatidylserine, chondroitin sulfate, vitamin E, and fish oil, all with small effect or not effective on DOMS. There is no clear consensus in the existing literature on an intervention that can effectively relieve pain after eccentric exercise. Conclusively, it is difficult to recommend with confidence the use of these methods for minimizing the signs and symptoms of muscle soreness.

Some herbs and spices such as saffron are widely used in the human diet. Saffron is a spice derived from the flower of the Crocus sativus. Saffron has been traditionally used in ancient medicine against various human diseases. Saffron stigma contains more than 150 volatile and aroma-yielding compounds. It also has many nonvolatile active components, many of which are carotenoids, including zeaxanthin, lycopene, and various α- and β-carotenes. Recent experimental findings indicate that saffron’s major compounds, crocin and crocetin, which are derivatives of carotenoids, are powerful antioxidants, with anti-inflammatory and antinociceptive activities.

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, indomethacin, and diclofenac are the most commonly investigated treatment for DOMS. The risk of overdose and its potential side effects such as stomach ulcers, hepatic, and renal toxicity should be considered. NSAIDs blocking cyclooxygenase enzyme thus inhibit the synthesis of prostaglandins from arachidonic acid and therefore neutrophil and macrophage functions are inhibiting. Previously, preventive effect of indomethacin (75 mg per day) for 5 days on the cytokine responses to strenuous exercise in humans has been studied. The results demonstrate that indomethacin blunted serum interleukin-6 and augmented tumor necrosis factor-alpha (TNF-α) and interleukin-10 during and after strenuous physical exercise. It is worthy to note that inflammation itself is an essential natural process of muscle remodeling and regeneration after muscle injury, and these medicines can cause harm to the muscle.

Based on the anti-inflammatory and antinociceptive effects of saffron and to compare any preventive effect of saffron with indomethacin, therefore, this study aimed to investigate the effects of 10-day supplementation with saffron in DOMS in young male university students.

METHODS

The Physical Activity Readiness Questionnaire (PAR-Q) was used for fitness and health screening of volunteers. Subjects were excluded if they regularly participated in vigorous exercise in the previous 3 months. Thirty-nine young healthy male nonactive university students (age: 18.2 ± 0.4 years) were selected among 50 volunteers. This study used a 10-day, randomized, double-blind, placebo-controlled, fixed-dose, parallel-group, pretest-posttest design. The university ethics committee approved the study (M416). The purpose and procedures of the study were fully explained, and they signed the consent form.

The weight of the subjects, wearing minimal clothing and standing height without shoes were measured with a Seca digital scale (Seca model 707; Vogel & Halke GmbH & Co, Hamburg, Germany) to the nearest 0.1 kg and 0.5 cm.

A dietary recommendation sheet was given to the subjects and requested to refrain from any nutritional supplements or medicines, including anti-inflammatory medicines, 1 week before and during the study. Crocus sativus L. stigma was powdered, and soft capsules were filled with 300 mg saffron powder. Contents of indomethacin capsules (25 mg) were transferred to a similar 500 mg capacity capsule, and identical placebo capsules containing 300 mg lactose were provided.

The saffron group received 1 capsule containing dried saffron stigma powder (n = 12, 300 mg/d), indomethacin group received 75 mg indomethacin (n = 12, 25 mg thrice a day), and the control group (n = 15) received placebo capsules containing 300 mg lactose powder. Participants were asked to take the saffron and placebo capsules daily at 6 PM with a cup of water and indomethacin every 8 hours immediately after a meal.

A leg press apparatus equipped with 2 force isometric sensors connected to a computerized dynamometer was used to determine the maximum isometric force. Participants were familiarized with the leg press machine and protocols before testing.

First, all the participants underwent a 10-minute warm-up. Then, each participant was tested for maximum isometric force in a leg press machine. The maximum isometric force was measured while the weight is locked and subject was seated on the leg press bench, placed feet on the plantar plate with knee angle at 90°. Two computerized dynamometer sensors measured the forces applied by the feet to the plantar plates. Force data were recorded on a laptop computer and software calculated the maximum isometric force during 3 to 5 seconds trial.

After 10-minute rest, maximum isometric force was measured when the weight bar was unlocked, and the subject was seated on the bench with knee angle at 90° and pushed-up the weights by feet. Verbal encouragement was given to achieve a maximal effort isometric and isotonic strength tests. After a 10-minute rest, participants were instructed to perform 4 sets of eccentric exercise on a leg press machine. The weight load was set to 80% maximum isometric force in 4 sets. Each set consisted of 20 repetitions with 3-minute rest between the sets. Participants were instructed to push the weights up by extending legs and let it slowly swing back after a short pause. Movements were harmonized by a metronome sound. The participants performed the protocol with a maximal effort until they were unable to continue a repetition with proper technique. The force applied to the plantar plate was recorded by a computerized dynamometer during the eccentric exercise. All participants completed the protocol.

One day before the supplementation and 3 days after eccentric exercise, the blood sample was taken after overnight fasting from the control and experimental groups and before other tests. Five milliliters of venous blood was sampled from...
RESULTS

The Shapiro–Wilk test shows normality of all the data distribution except for the pain perception in the pretest due to pain-free status. Interpretation of data was performed using a 2-way repeated-measure ANOVA with Bonferroni pairwise comparison. Results obtained from the repeated-measure ANOVA with Bonferroni pairwise comparison were used for interpretation.

The maximum isometric force showed equality in the maximum force between all groups in the pretest session. Conspicuously, in the control group, there was a significant decline in the maximum isometric force: after 24 hours (15.3%), 48 hours (23.8%), and 72 hours (24.3%) after the eccentric protocol. The maximum isometric force between saffron and control groups showed significant differences after 24 ($P < 0.05$), 48 ($P < 0.0001$), and 72 hours ($P < 0.0001$). The indomethacin and control groups comparison had a significant difference after 48 ($P < 0.05$) and 72 hours ($P < 0.05$). Comparison between saffron and indomethacin showed the only significant difference after 24 hours ($P < 0.05$). The effect size between saffron and control group after 24, 48, and 72 hours was 0.99, 1.7, and 2.1, respectively. The effect size between indomethacin and control group after 24, 48, and 72 hours was 0.02, 0.19, and 0.23, respectively. The saffron group had the highest effect size after 72 hours of muscle soreness (Cohen’s $d = 2.149$) (Figure 1).

At baseline, there were no significant differences in maximum isometric force between groups. The saffron group exhibited significant increase in the isometric force after 24 ($P < 0.05$), 48 ($P < 0.05$), and 72 ($P < 0.0001$) hours (63.6% increase) compared with baseline. The maximum isometric force between saffron and control groups showed significant differences after 24 ($P < 0.05$), 48 ($P < 0.0001$), and 72 hours ($P < 0.0001$). Unlike saffron group, the control group showed a significant decrease in the isometric and isotonic forces. Comparison between saffron and indomethacin showed a significant difference only after 72 hours ($P < 0.05$), whereas the indomethacin and control groups comparison had a significant difference after 48 and 72 hours ($P < 0.05$). The effect size between indomethacin and control group after 24, 48, and 72 hours was 2.6, 2.9, and 2.6, respectively. The effect size between indomethacin and control groups after 24, 48, and 72 hours was 1.3, 0.28, and 0.29, respectively. The highest effect size belonged to the saffron group compared with control after 72 hours of muscle soreness (Cohen’s $d = 2.9$) (Figure 2).

Plasma CK values were not significantly different between the groups at baseline. However, Figure 3 demonstrates that after 24 hours, CK concentration in the saffron and indomethacin groups decreased as compared with the control group. Plasma CK in the control group peaked at 48 hours after exercise (125.8%). Plasma CK level in the saffron group compared with baseline (100%) increased after 24 hours of exercise but it was not significant (3.7%) after 24 hours of exercise and then returned to a normal level. Plasma CK in saffron and control groups was significantly different after 24, 48, and 72 hours ($P < 0.0001$). However, a difference between indomethacin and control groups was significant only after 48 and 72 hours ($P < 0.0001$). Saffron and
indomethacin groups exhibited a significant difference only after 24 hours \((P < 0.005)\). The effect size between saffron and control groups after 24, 48, and 72 hours was 2.6, 2.9, and 2.6, respectively. The effect size between indomethacin and control groups after 24, 48, and 72 hours was 1.3, 0.28, and 0.29, respectively. The maximum effect size has been seen between saffron and control groups after 24 hours (Cohen’s \(d = 3.397\)).

There was no significant difference between groups in the plasma LDH concentration at baseline. Plasma LDH concentrations presented in Figure 4 indicate that the saffron group exhibits a significant decrease after 24, 48, and 72 hours \((P < 0.0001)\). The indomethacin group has a significant difference with control group after 48 and 72 hours \((P < 0.0001)\). The saffron and indomethacin comparison had a significant difference only after 24 hours.
The effect size between saffron and control groups after 24, 48, and 72 hours was 2.6, 3.8, and 2.7, respectively. The effect size between indomethacin and control group after 24, 48, and 72 hours was 2.2, 0.61, and 0.63, respectively. Highest level of effect size was observed in the comparison between saffron and control groups after 48 hours (Chohen’s $d = 3.772$).

Right leg pain perception had a significant difference between saffron and control groups after 24, 48, and 72 hours ($P < 0.0001$) (Figure 5). Pain perception in the saffron group after 24 hours was 11.2 times lower than the control group, and all the subjects in the saffron group experiences no pain after 48 and 72 hours, whereas in the indomethacin group pain alleviated after 72 hours.

FIGURE 3. Comparison between plasma CK concentration in the saffron (♦), indomethacin (■), and control (▲) groups (mean ± SD). *Indicates a significant difference ($P < 0.0001$) compared with the control group.

FIGURE 4. Comparison between plasma LDH concentration in the saffron (♦), indomethacin (■), and control (▲) groups (mean ± SD). *Indicates a significant difference ($P < 0.0001$) compared with the control group.
The right thigh circumference did not show any significant differences at baseline. In the control group, the thigh circumferences after 24, 48, and 72 hours of eccentric exercise showed a significant increase as compared with the pretest. Thigh circumference in the saffron group did not increase after eccentric exercise. There was no significant difference between the indomethacin and control groups (Table).

Control group exhibited a significant decrease in the right knee joint ROM 24, 48, and 72 hours after exercise compared with the pretest. The right knee joint ROM in saffron and control groups had no significant differences after 24, 48, and 72 hours. However, comparison between indomethacin and control groups showed a significant difference after 24 hours ($P < 0.0001$).

**DISCUSSION**

The present investigation demonstrated that saffron and indomethacin markedly prevented the laboratory and clinical indices of exercise-induced DOMS in the nonactive young university students. The saffron was also more effective compared with indomethacin in the prevention of DOMS. The results of this study are encouraging because they serve to highlight the potential importance of saffron as a natural product in aiding the performance of athletes.

The best indicator of muscle damage is the force loss.\(^{14}\) In our experiment, the control group had a significant decrease in the maximum isometric and isotonic forces through the study. Noticeably, isometric force in the control group significantly decreased (19.6%), whereas saffron group experienced a significant increase after eccentric exercise (63.6%). However, there were nonsignificant changes of maximum isometric force in the indomethacin group. This tendency might provide a clue that saffron has double positive effects on muscle, the preventive effect on DOMS, and muscle force enhancement.

It has been proposed by Proske and Morgan\(^ {55} \) that muscle damage due to eccentric exercise leads to a release of $\text{Ca}^{2+}$.

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**TABLE.** Participants’ Range of Motion of the Knee and Thigh Circumference Measurements Between the 3 Groups During the Tests (Mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>24 Hours</th>
<th>48 Hours</th>
<th>72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thigh circumference</td>
<td>Saffron</td>
<td>53.0 ± 4.91</td>
<td>53.0 ± 4.87</td>
<td>53.0 ± 4.91</td>
</tr>
<tr>
<td></td>
<td>Indomethacin</td>
<td>54.1 ± 4.70</td>
<td>54.7 ± 4.72</td>
<td>54.0 ± 4.70</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>53.8 ± 4.83</td>
<td>56.2 ± 5.15*</td>
<td>57.1 ± 4.81*</td>
</tr>
<tr>
<td>Knee ROM</td>
<td>Saffron</td>
<td>41.1 ± 3.39</td>
<td>41.0 ± 3.39</td>
<td>40.8 ± 3.35</td>
</tr>
<tr>
<td></td>
<td>Indomethacin</td>
<td>35.2 ± 2.5</td>
<td>36.1 ± 2.51†</td>
<td>35.2 ± 2.52</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>36.7 ± 2.0</td>
<td>39.0 ± 2.07*</td>
<td>41.0 ± 2.18*</td>
</tr>
</tbody>
</table>

*Significant difference $< 0.001$.
†Significant difference $< 0.0001$. 

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in the sarcoplasm. The increased Ca\textsuperscript{2+} levels can trigger proteolysis and assist in the break down of the damaged muscle fibers. Moreover, damage due to increased myoplasmatic Ca\textsuperscript{2+} levels can lead to inflammation, resulting in edema and muscle swelling.\textsuperscript{55,56} The inflammation is assumed to sensitize the nociceptors and leads to pain in the affected area.\textsuperscript{57,58} Our previous report about the novel protective effect of saffron on the erythrocyte osmotic hemolysis (76.0%)\textsuperscript{59} might support this notion that saffron might protect the muscle fibers by enhancing the muscle cell membrane integrity.

An increase in the maximum isometric force in the saffron group is a novel finding of this study. In a very recent and unpublished research, we found significant ergogenic effect of saffron after 10-day saffron supplementation. We postulated that this ergogenic effect of saffron might be related to (1) enhance muscle metabolism, (2) increased neuronal excitability, increased motor unit recruitment, (3) combination of these mechanism with or without anti-inflammatory and antinociceptive effects of saffron. Antioxidant effects and the facilitation in tissue oxygenation properties of saffron components (eg crocin, crocetin, and safanal) were previously reported.\textsuperscript{60}

Increases in plasma CK and LDH levels are established indicators of inflammation and pathological consequences of DOMS. Saffron showed better prevention of CK and LDH elevation after eccentric exercise compared with indomethacin. Anti-inflammatory and antioxidant effects of saffron are the most likely responsible. These effects may also be responsible for the perceived pain tolerance in this study. This study was limited to the inactive and healthy university male students, and similar research on the athletes is necessary to find out the effectiveness of saffron on the athletes.

CONCLUSIONS

We conclude that 10-day supplementation with saffron has potent preventive effects on DOMS as compared with indomethacin and control groups. The ancient spice saffron has shown eminent preventive effect on DOMS as compared with indomethacin. Considering NSAIDs medicine side effects, saffron with its health-promoting properties will be preferred in the prevention of DOMS. Further research is necessary to unravel the mechanisms involved and dose-dependent effect of saffron on DOMS.

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REFERENCES


